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Trend analysis of performance parameters of pre-packed columns for protein chromatography over a time span of ten years

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ABSTRACT

Pre-packed small scale chromatography columns are increasingly used for process development, for determination of design space in bioprocess development, and for post-licence process verifications. The packing quality of 30,000 pre-packed columns delivered to customers over a period 10 years has been analyzed by advanced statistical tools. First, the data were extracted and checked for inconsistencies, and then were tabulated and made ready for statistical processing using the programming language Perl (https://www.perl.org/) and the statistical computing environment R (https://www.r-project.org/). Reduced HETP and asymmetry were plotted over time to obtain a trend of packing quality over 10 years. The obtained data were used as a visualized coefficient of variation analysis (VCVA), a process that has often been applied in other industries such as semiconductor manufacturing. A typical fluctuation of reduced HETP was seen. A Tsunami effect in manufacturing, the effect of propagation of manufacturing deviations leading to out-of-specification products, was not observed with these pre-packed columns. Principal component analysis (PCA) showed that all packing materials cluster. Our data analysis showed that the current commercially available chromatography media used for biopharmaceutical manufacturing can be reproducibly and uniformly packed in polymer-based chromatography columns, which are designed for ready-to-use purposes. Although the number of packed columns has quadrupled over one decade the packing quality has remained stable.

1. Introduction

Pre-packed columns or ready-to-use laboratory chromatography columns have been on the market for about one decade and have become very popular for process development and *a posteriori* evaluation of design space [1,2]. Such columns are used to reduce time for packing, which can be very tedious. Time savings is the major criterion for why pre-packed columns are widely applied in biopharmaceutical industry. It is assumed that the performance does not change over time and that consistent lots can be produced. These columns are often used to corroborate findings which have been made with automated systems either by parallel chromatography in robotics systems [3] or by adsorption measurements in microtiter plates [4–6]. For ready-to-use disposable columns, column construction must be simple and inexpensive. Adjustable pistons like adaptors are too expensive for this purpose. Therefore, a precise amount of chromatography medium must be packed into the column, which requires knowledge and skill in column packing. The performance of the packed columns is also checked by the manufacturer before sale. The customers assume that all ready-to-use columns display the same packing quality and can be applied without re-checking the packing quality. Interestingly, the quality can change to certain extent over time.

Manufacturing systems can be divided into 4 major models, transformation operations, operations of modification of structure, information operations, and transfer operations [7]. We can also assume that the same principles of manufacturing variations apply to column packing as used in other industry areas. Column packing can be considered as a transformation operation.

In principle, chromatography media can be categorized according to the backbone: (1) natural polymers such as agarose or

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dextran; (2) synthetic polymers such as acrylamide or polymethacrylate; and (3) inorganic materials such as silica, hydroxy apatite, or controlled pore glass [8]. Many methods have been described for column packing taking into account the nature of the backbone. The influence of packing procedures on packing quality of small columns is still not fully understood [9]. Semi-rigid chromatography material is easier to pack than rigid particles and soft material such as Sephadex G-25 or Biogel [10–12]. Slurry packing under flow (flow packing) is routinely used for packing of chromatography material based on soft natural and synthetic polymers. The column is packed under a higher flow rate and pressure than applied during separation. Rigid particles are often dry-packed, but this depends on the particle size. The beads are filled into the column and are then compressed, a process also known as axial compression [13–17]. Such axial compression can be also applied for slurry packing, but is not feasible for ready-to-use columns because a piston is not part of the construction. Vibration of the column during packing may help to improve packing density. This technique has been successfully practiced for many years [18–20].

The standard parameters for controlling the packing quality of columns on all scales are HETP (Height Equivalent to a Theoretical Plate) and asymmetry [21,22]. HETP is a parameter which is independent of column length but depends on size of the particle, pore size, pore size distribution, particle porosity, velocity and the solute which is used for the experiment. When material dedicated for protein solution is tested with a small molecule tracer then the pore, pore size, pore size distribution and particle porosity is of less concern, because the molecules have a very high effective diffusivity. Asymmetry is another very good parameter to quantify the packing quality because it indicates if the packing density close to the column wall is lower or higher than in the center of the column. It is also a measure of the exponential wash out caused by extra column volume [23]. The experimental conditions to measure HETP and asymmetry must be standardized with respect to applied solute and velocity. It is also well known that the method of peak fitting may influence the outcome, e.g., graphical peak integration, numerical integration or fitting of the peak by a model function and calculation of variance and retention time based on the model. In addition to the experimental conditions, the data evaluation must be highly standardized in order to compare packing qualities among different experiments [24,25].

In order to see trends in a material property, a simple trend analysis can be performed. A simple but very effective way is to plot the property over time to visualize changes. The variation of the data can be assessed by, e.g., principal component analysis, which may help to identify co-variances. We received the performance data of 30,000 packing experiments of ready-to-use columns. Different chromatography media designed for purification of proteins and other large biomolecules were packed under standardized conditions. For each medium, an optimal procedure was developed by the column packing company. The packing quality was tested by injection of an aliquot of acetone and the retention time and peak width were determined from the peak profiles. The same procedure was used over the entire production period of approximately ten years. The injection of a small tracer molecule was selected since due to the large pores, only the hydrodynamic dispersion was measured. This dispersion is only influenced by the packing quality and the extra-column dispersion including the dispersion by the header and adapter.

An enormous challenge of this study was the extraction of the huge quantity of experimental data obtained from column testing. A proprietary closed-source software (Eurochrom) had been used to originally test the columns and to store the corresponding results. Eurochrom stores the data in a binary format and does not provide an API (application programming interface) that enables accessing the data via another program. Consequently, the column data could only be retrieved via Eurochrom's GUI (graphical user interface). For a small number of samples, a manual export is possible. However, the manual data export of 30,000 test runs which had been stored in 550 Eurochrom databases spread over 535,000 binary files was a very time consuming and error-prone process. Therefore, a computer software program was written in the programming language Perl with the module Win32:GuiTest which emulated mouse events and key strokes of a human computer user and utilized Eurochrom's GUI to automatically retrieve the column test data that had been generated over a period of ten years. After extraction, the data were read into the statistical computing environment R (https://www.r-project.org/) where the data was further processed, summarized, and visualized. Principal component analysis [26] was used to find the directions of the largest variations of the data, to visualize present structure in the data, and to detect outliers. This paper provides a useful and practical example of how preparative chromatography with ready-to-use columns can be standardized.

2. Materials and methods

2.1. Chromatography workstation and column packing

A chromatography workstation from Knauer, Berlin, Germany was used. The workstation was controlled by the Eurochrom software, which also handled data storage and peak analysis. Columns were packed by slurry packing under vibration.

2.2. Determination of HETP

HETP was measured by injection 50 μl of acetone or sodium nitrate and the UV 218 nm response was recorded. The chromatographic workstation automatically determined the number of plates N. From retention time (t) and N ($N = 5.54^{*} (t/W_{0.5})^2$) determined from the peak width measured at half peak height ($W_{0.5}$), the peak width (σ) was calculated by

$$\sigma = \sqrt{\frac{t^2}{N}} \tag{1}$$

An effective plate number was used for the data evaluation

$$N_{eff} = \left(\frac{t - t_0}{\sigma}\right)^2 \tag{2}$$

where t is the retention time, t_0 the dead time and σ^2 the variance.

Height equivalent to one theoretical plate (*HETP*) is defined as

$$HETP = L\frac{\sigma^2}{\mu^2} \tag{3}$$

with σ^2 the variance, L the column length, and μ the first peak moment. Reduced HETP (h) is obtained by dividing HETP by the particle diameter (d_p).

$$h = \frac{HETP}{d_p} \tag{4}$$

Asymmetry (A_s) was calculated at 10% peak height [27] with

$$A_s = \frac{b}{a} \tag{5}$$

a the width of the front part of the peak divided at peak maximum and b the width of the rear part.

Reduced velocity was calculated as

$$v = \frac{u \cdot d_p}{D_0} \tag{6}$$

with u the linear velocity, and D_0 the molecular diffusivity of the tracer molecule.

2.3. Data extraction

Test runs of approximately 30,000 columns were performed over a period of ten years using the proprietary closed-source software Eurochrom by Knauer GmbH. The Eurochrom software only runs on Microsoft operating systems (Windows XP and Windows 2000) and is no longer maintained. All column data records were stored in Eurochrom databases. Eurochrom databases use binary files to persistently store and retrieve data of the tested columns. All binary files belonging to a single database are located within a single directory. The name of a directory is identical to the database name. In total, 550 databases comprised approximately 535,000 binary files. The format specification of Eurochrom's binary files was not available.

Hence, we implemented a computer tool in the programming language Perl (https://www.perl.org/) which emulates a human computer user. Perl is a freely available high-level programming language that runs on all relevant operating systems, such as Linux/Unix, Mac OS, and Microsoft Windows. We used Strawberry Perl (http://strawberryperl.com) for this study. The implemented Perl program controls the input devices (keyboard and mouse) of the computer and is, therefore, able to perform any task that a human user can do. The core element of our program was the Perl module Win32:GuiTest which is available at CPAN Comprehensive Perl Archive Network (http://www.cpan.org/). CPAN is a huge repository of Perl modules that contains more than 150,000 freely available Perl modules.

The principle workflow of our program was as follows:

- Loop over all 550 Eurochrom databases (directories on Windows file system)
  - Start Eurochrom software
  - Open current database
  - Loop over all test runs of current database
    - Retrieve data from database for current test run
    - Export general information about test run (Report)
  - Close current database
  - Stop Eurochrom

The final output of our program was 30,000 exported report files and took approximately six days to complete. The output files of our program were simple plain text files which (a) can be edited by any text editor and (b) can be easily processed by any scripting programming language, such as Perl or Python. In a post-processing step, the 30,000 data files were aggregated to a single text data file. During this aggregation process the data records were checked for plausibility and any invalid data record was removed before the statistical analysis was started.

Emulating a human user is an extremely challenging task. This is especially true if the emulator has to react to unexpected events. For instance, dialog windows which might pop up at any time to inform the user about available security updates can easily result in a malfunction that (a) stops the automatic process of data extraction and (b) in the worst case, can irreparably damage the computer system by – for instance – accidentally deleting essential files. In order to avoid any unwanted side effects, we took the following measures: (i) uninstalled any software not required for the extraction of the column data; (ii) disabled all automatic notifications; (iii) implemented a feature that tracked the successfully exported databases and allowed us to restart the extraction process at the point where an error has occurred; and (iv) ran the data extraction on a virtual machine. A virtual machine has the advantage that the entire file system of the virtual machine is a single file on the host machine. Hence, backing up and restoring a virtual machine can simply be done by copying a single file on the guest machine. We used VirtualBox (https://www.virtualbox.org/) by Oracle for our virtualization approach. The virtual machine was running Windows XP and the host machine used a Linux Ubuntu 14.04.

Table 1
Summary of top 30 chromatography material studied.

Name	Counts	Functional mode
MEP HyperCel™	1442	Mixed
HEA HyperCel™	984	Mixed
PPA HyperCel™	946	Mixed
PROSEP® Ultra Plus	828	Affinity
Q Ceramic HyperD® F	812	Hydroxapatite
Q HyperCel™	775	AIEX
S HyperCel™	749	CIEX
MabSelect SuRe™	703	Affinity
Capto™ L	614	Affinity
Fractogel® EMD SO3- (M)	598	CIEX
UNOsphere™ Q	524	AIEX
Eshmuno® A	498	Affinity
Fractogel® EMD COO- (M)	495	CIEX
CM Ceramic HyperD® F	453	CIEX
Fractogel® EMD TMAE (M)	448	AIEX
Fractogel® EMD TMAE Hicap (M)	443	AIEX
Strep-Tactin® Superflow®	428	Affinity
HyperCel™ STAR AX	422	AIEX
Fractogel® EMD DEAE (M)	421	AIEX
Eshmuno® Q	413	AIEX
Fractogel® EMD SE Hicap (M)	403	CIEX
Eshmuno® CPX	398	CIEX
Fractogel® EMD DMAE (M)	367	AIEX
MabSelect™	365	Affinity
Strep-Tactin® Superflow® high capacity	347	Affinity
Eshmuno® S	344	CIEX
Eshmuno® HCX	336	AIEX
POROS® 50 HS	262	CIEX
Macro-Prep® CHT™ Type I 40 μm	260	Hydroxapatite
ProSep® Ultra Plus	257	Affinity

2.4. Data analysis

The data were read into R where duplicate entries and entries with missing information were removed. After these polishing steps, the data contained 24,951 chromatography runs. The available variables included time, chromatography material, particle size, column diameter and length, retention time, injection time, number of plates, and asymmetry. Additional variables such as reduced HETP and reduced velocity were calculated. Particle diameters were obtained from the manufacturer's information of the individual media. The data were summarized and visualized for each particle size, backbone, functional mode, and size of the columns. The 30 most frequently used chromatography materials in this study are listed in Table 1. The Supplementary material Table sub1 shows all materials included in the data.

A mosaic plot is a graphical display that allows one to examine the relationship among two categorical variables. The mosaic plot starts as a square with unit length one. The square is divided first into horizontal bars whose widths are proportional to the probabilities associated with the first categorical variable, in this case year. Then each bar is split vertically into bars that are proportional to the conditional probabilities of the second categorical variable, i.e., backbone and functional mode of the chromatography materials.

The size of the tiles is proportional to the cell frequency, i.e., the materials per categories, which have been tested per year. The cells are shaded in proportion to standardized residuals from the log-linear model that year and category (backbone and functional mode) are independent in this dataset, which is, of course, not the case. Thus, tiles shaded in dark blue are significantly larger than

expected whereas tiles shaded in dark red are significantly smaller than expected.

2.5. Principal component analysis

In principal component analysis (PCA) [26], a set of possibly correlated variables is converted by an orthogonal transformation to a set of uncorrelated variables called principal components. The number of principal components is less than or equal to the number of original variables. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component, in turn, has the highest variance possible under the constraint that it is orthogonal to the preceding components. Here PCA was used to find directions of the largest variations of the data, to visualize present structures in the data, and to detect outliers.

3. Results and discussion

After data extraction and checking for inconsistencies, exploratory data analysis was performed. A trend analysis was generated showing the reduced HETP (h) of all chromatography materials irrespective of functionalization and backbone over a period of ten years (Fig. 1). The values of h were always in the range of 5 with slight variations over time and a decrease in the year 2012 and 2013. Data collection was stopped after January 2015. To prevent the possibility that the frequency of the individual chromatography materials within a certain time period could influence h, mosaic plots were generated.

We did not observe a clear trend that a single functionality was packed particularly often (Fig. 2A). However, we were able to detect several other trends. The most material was packed in 2013 and 2014 whereas in 2007 the least amount of material was packed. In the first years, lesser amounts of AF, AIEX and CIEX materials were packed whereas more HCIC and MMC materials were packed. The mosaic plot of materials according to backbone also showed trends (Fig. 2B). Compared to the other resin types, agarose-based materials were less frequently packed in the first 4 years and in the last year. Cellulose-based resins were packed more frequently in the first 4 years and polymethacrylate and poylvinlyether-based materials were increasingly applied during later years. Inorganic material was frequently packed in the beginning and then only rarely thereafter.

We then investigated if different categories showed differences in h in order to determine if the inhomogeneous frequency of materials influenced the data. Plotting h versus particle size allowed us to evaluate the quality of packing (Fig. 3A and B). Values of h did not depend on the particle size over a wide range, because theoretically one should obtain the same value assuming the reduced velocity (ReSc) is the same for all runs. In the present study, experimental conditions for measurement of HETP were selected in a way that the results can be expected to fall within the hydrodynamic dispersion region, v < 15, and not in the mass transfer limited region, v > 100 (Fig. 4) [28]. The pore size was at least 100 times larger than the size of the tracer molecules acetone or sodium nitrate, because all tested media are intended for separations of proteins and other large biomolecules. The velocities were in a moderate range so that a low reduced velocity can be expected. The same solute was used for one resin over the entire time period and the peak integration method was always identical. Thus, two major sources of error due to varied experimental set-up or data treatment can be excluded and observed variations can be assigned to packing quality only. The obtained results indicated that the smaller particles were more difficult to pack (Fig. 3A). It is a compromise to pack small particles in a ready-to-use column. A relevant difference cannot be observed for particles larger than 50 μm although all media showed significantly different values of h. This is simply explained by the large data set and because h is a unique feature of each chromatography material (Fig. 3). Resins with a particle diameter larger than 50 μm displayed values of h smaller than 5, except for 51.5, 74.5 and 136.5 μm particles, which were all Sephadex G-25 products. Sephadex G-25 is a very soft material with a substantial bed compression. This may be an explanation for this behaviour. Slightly different packing procedure has been proposed, but this method cannot be implemented in a large scale high throughput industrial packing line [10].

For the intended purpose of protein chromatography, the columns can be considered as well packed when h is in the range between 3–15 as shown in Fig. 1. For the actual experiments with proteins and other large biomolecules the columns will be operated in the mass transfer limited regime, where reduced velocity v is >100 and reduced HETP h for this class of molecules is >100. This is exemplified in Fig. 4 where the data are plotted together with reduced HETP h for proteins. The presented data for proteins have either been taken from the literature or from our own experimental results. Although columns can be considered as well packed for the intended purpose, the trends were investigated in more detail according to the categories of chromatography media sorted by their modes of functionalization (Fig. 5). The hydrophobic or electrostatic character of the beads may prevent a full consolidation of the bed to which extent the functionaliztation has an effect is not fully understood [29]. It is well understood that tamping or vibration improves bed consolidation [19,20]. This practice has been applied for all media, but dynamic axial compression cannot be applied for these small ready-to-use columns. The ready-to-use columns are not intended for high resolution separation, most frequently they are used for confirmation of screening results obtained with even smaller columns or batch adsorption studies made, for instance, in microtiter plates. Therefore, the determined packing quality of these ready-to-use columns serves the intended purpose. To gain greater insight, the data were also categorized according to the nature of the backbone. For each category of backbone, a much more detailed picture was obtained than when all packing data were pooled and analyzed. The backbone gave the biggest contribution to the packing quality. Although not fully understood, the density and surface roughness of the chromatographic media is determined by the nature of the backbone which can be composed of inorganic material, natural polymer, and organic polymer. Analyzing media of different backbone materials but the same functionalization (Fig. 5A) provided a more heterogeneous picture as compared to media of the same backbone differing in functionalization (Fig. 5B). Hydroxy apatite (HA) is difficult to pack [30], thus the variation in reduced HETP h is large but still within an accept-

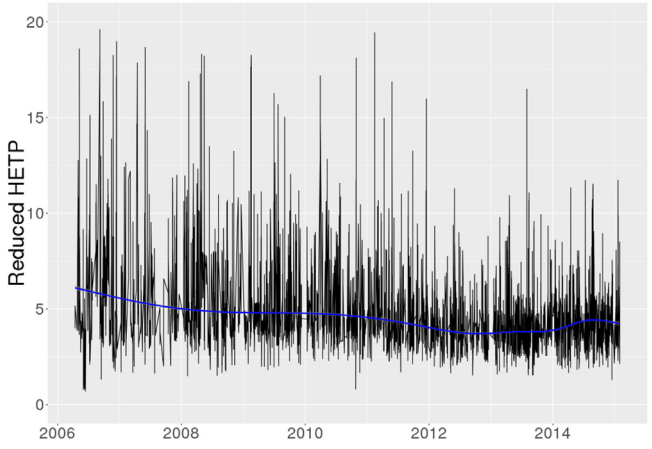

Fig. 1. Trendline of 24,626 ready-to use columns over a time span of 10 years. 325 columns with reduced HETP larger than 10 were not displayed in this figure.

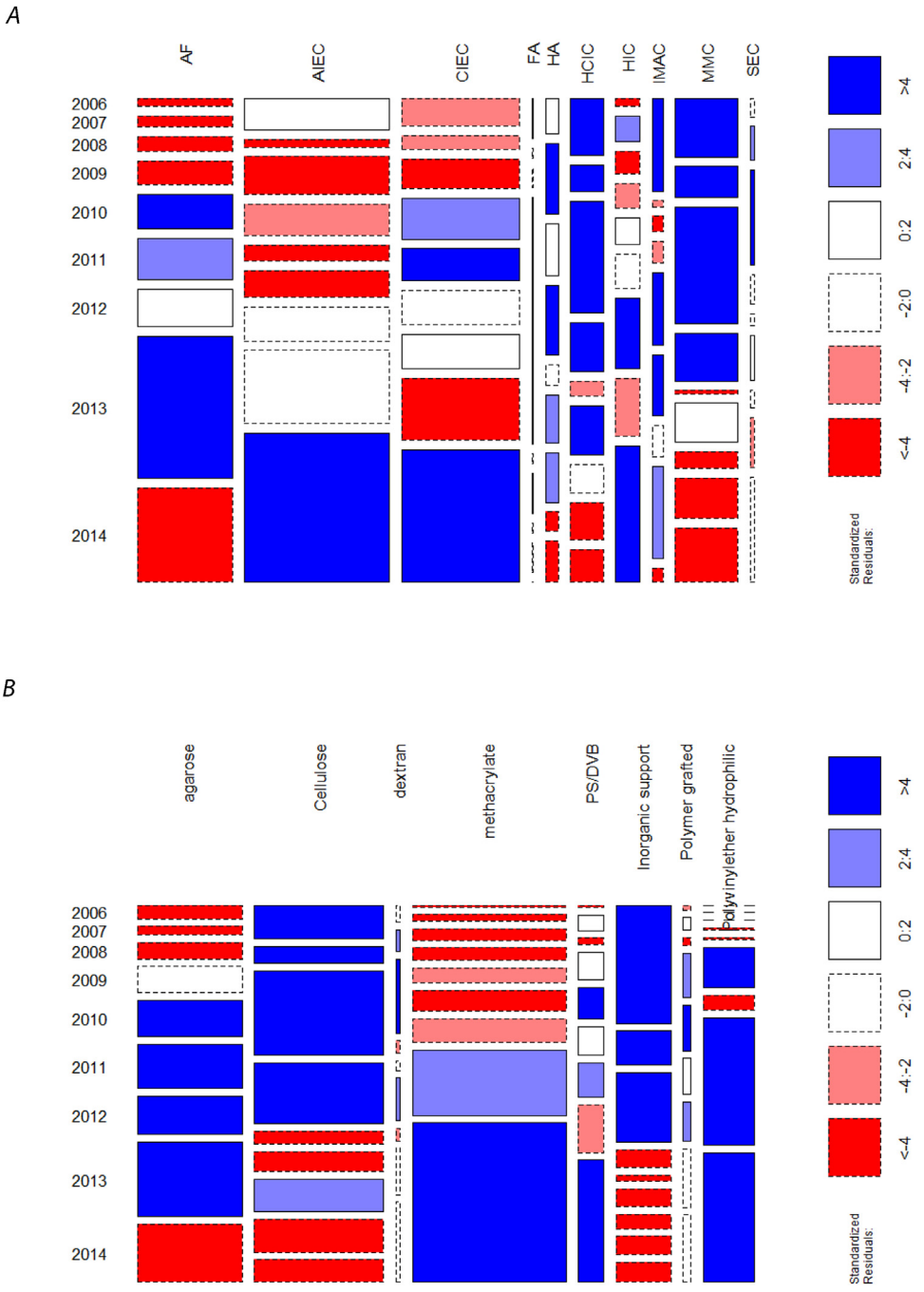

Fig. 2. Contingency table by year presented as a Mosaic plot categorized according to (A) functional chromatography modes and according to (B) composition of the backbone. The size of the tiles is proportional to the cell frequency, i.e., the materials per categories, which have been tested per year. The cells are shaded in proportion to standardized residuals. Tiles shaded in dark blue are significantly larger than expected whereas tiles shaded in dark red are significantly smaller than expected. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

able region for protein chromatography (Fig. 5A). Sorting all data by functionalization and year revealed a similar pattern for each year (Supplementary material Fig. sub2). This observation corroborated the trend-line shown in Fig. 1. We also plotted the reduced HETP (h) in relation to column volume, to examine if column size had an effect on packing quality (Fig. 6). No trend was observed; h did not change with column size.

A trend line was also generated for the asymmetry (Fig. 7) showing that this performance parameter was stable over the past ten years. The asymmetry is influenced by extra column band spreading and thus is influenced by the size of the column. The asymmetry clearly decreased with increasing column volume (Fig. 8). This effect could be caused by either extra column effects or because small columns are more difficult to pack. We have previously shown that up to 90% of the peak broadening in small columns is generated by extra column effects [23].

Principal component analysis was applied to investigate the structure of the data. In order to reduce the huge data size of almost 25,000 datapoints all (1116) unique resin-diameter-length combinations were formed and mean values for reduced HETP (h) and asymmetry were used as metric parameters in PCA together with particle size, column diameter and length. A biplot (Fig. 9) presents both the observations and variables of a matrix of the multivariate data on the same plot. The data are projected on two principal com-

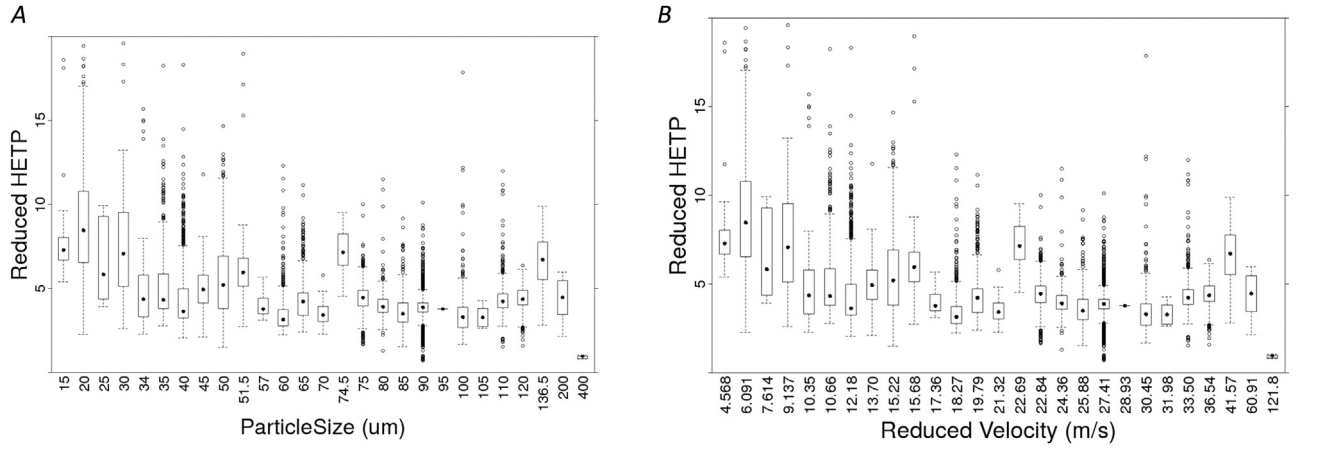

Fig. 3. Box and Whisker Plot for h versus particle size (A) and h versus reduced velocity (B); note that the distances between the particle sizes and reduced velocities are not equal.

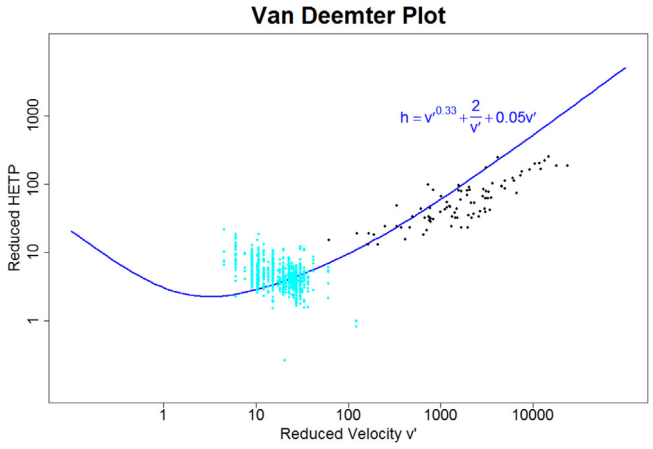

Fig. 4. Reduced HETP of acetone and selected examples for reduced HETP of proteins. The curve shown in blue is the generalized Van Deemter curve. The analyzed data set using a small molecule as tracer is shown in turquois and reduced HETP data for proteins are shown in black. These dots were reconstructed from the literature and from experiments in our laboratory and were measured for various resins (Source 30S, SP Sepharose FF, Fractogel EMD, POROS 50 HS, POROS Q/M, CaptoS and UNOSphereS) and proteins (lysozyme, bovine thyroglobulin, immunoglobulin G, myoglobulin, ovalbumin, bovine serum albumin, human serum albumin and cytochrome c). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

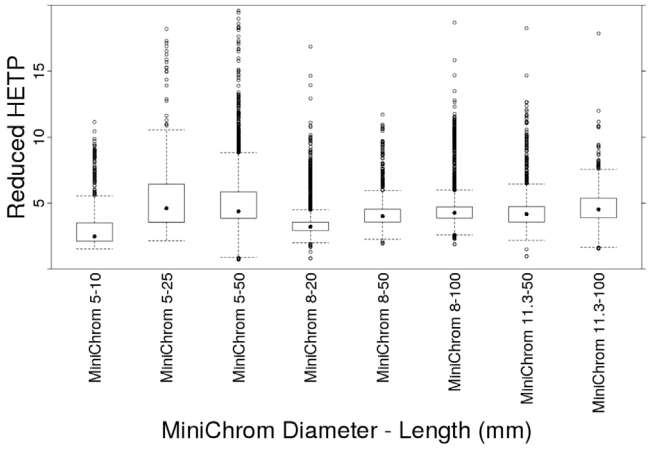

Fig. 6. Reduced HETP by the size of the columns.

ponents (PC), in this case the first and second PC are used, which accounts for 64% of the variance explained. The direction of the variables, here reduced HETP (h), asymmetry, particle size, column length, and diameter, are given by the blue arrows. The farther away from the center an observation is located, the larger the impact of the closest variable on the observation. The observations are all placed in one big cloud with single outliers in the directions of all

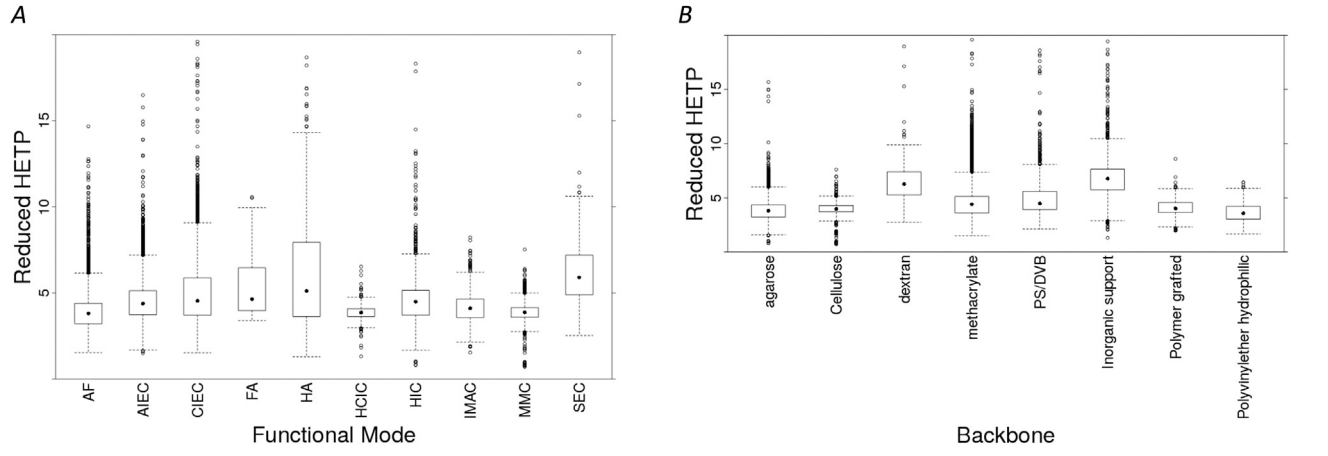

Fig. 5. Trend Analysis Boxplots of Reduced HETP by Functional Mode.

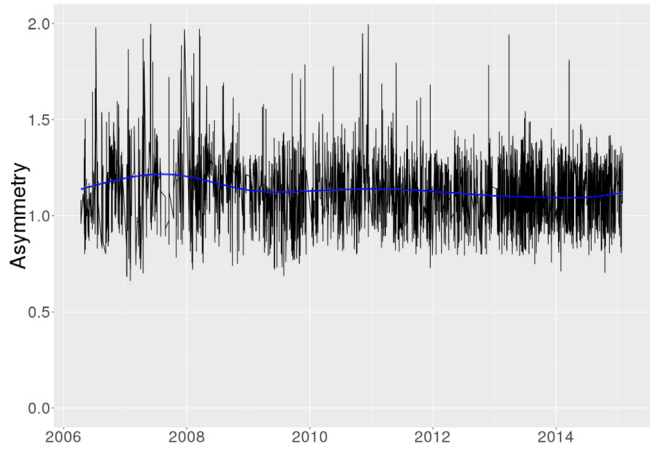

Fig. 7. Trend Analysis of Asymmetry (Tailing factor) over time.

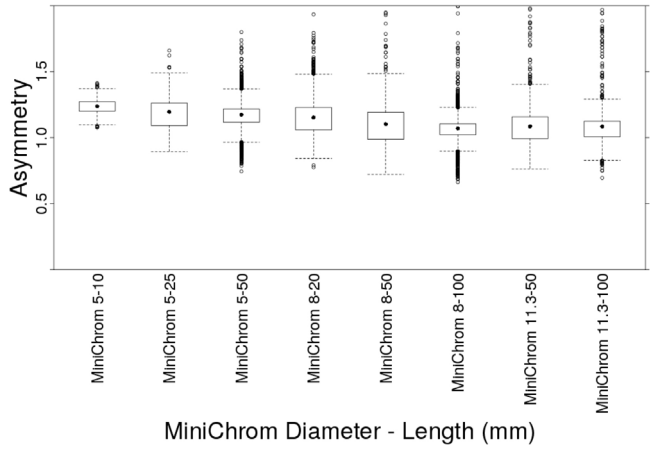

Fig. 8. Asymmetry plotted versus the size of the columns.

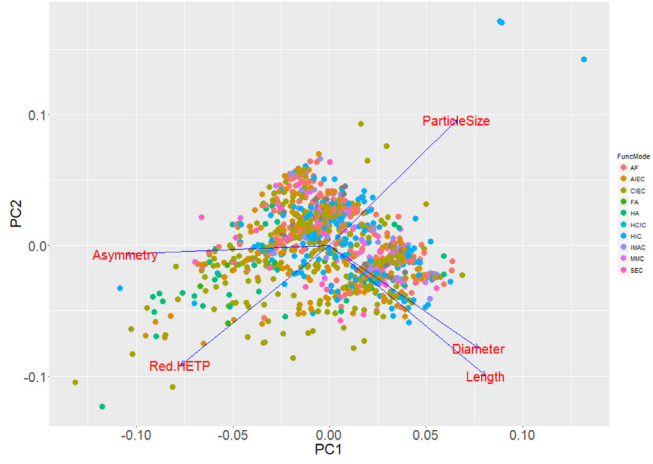

Fig. 9. PCA to identify variables influencing quality of packing. The directions of the variables reduced HETP (h), asymmetry, particle size, column length, and diameter, are given by the blue arrows. The left and bottom axis show the data projected to the first and second principal components (PC1 and PC2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

variables. This PCA showed that all packing materials clustered very well.

Theoretical analysis from Schure and Maier [31] showed that the column packing procedure must prevent the formation of defective sites leading to inhomogeneous packing rather than aiming for the highest packing density. Guiochon and colleagues concluded that a "well packed column is not optimal packed, it is reproducible" [32]. In this respect, the investigated large number of columns were well packed over an impressively long period of time, 25,000 columns over ten years.

4. Conclusion

The statistical evaluation of the quality parameters of reduced HETP and asymmetry of 25,000 different ready-to-use columns showed they were well packed. Variations in reduced HETP were higher than variations in asymmetry, which is influenced by the column geometry and design. The functional mode did not influence the packing quality. However, we observed a connection between packing quality and the composition of the resin backbone. Asymmetry slightly decreased with increasing size of the column. For their intended purpose as ready-to-use columns, reproducible packing quality has been observed over one decade, since these types of columns became commercially available.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.07.054.

References

[1] V. Brenac Brochier, H. Chabre, A. Lautrette, V. Ravault, M.N. Couret, A. Didierlaurent, P. Moingeon, High throughput screening of mixed-mode sorbents and optimisation using pre-packed lab-scale columns for the purification of the recombinant allergen rBet v 1a, J. Chromatogr. B 877 (2009) 2420–2427.
[2] J. Friedle, Chromatography media scouting, Eur. Biotechnol. Sci. Ind. News 7 (2008) 41–42.
[3] T. Schroeder, Automated parallel chromatography in downstream process development, Bioprocess Int. 8 (2010) 92–93.
[4] K.M. Łacki, High-throughput process development of chromatography steps: advantages and limitations of different formats used, Biotechnol. J. 7 (2012) 1192–1202.
[5] X. Li, G. de Roo, K. Burgers, M. Ottens, M. Eppink, Self-packed filter plates: a good alternative for pre-packed filter plates for developing purification processes for therapeutic proteins, Biotechnol. J. 7 (2012) 1269–1276.
[6] N. Sanaie, D. Cecchini, J. Pieracci, Applying high-throughput methods to develop a purification process for a highly glycosylated protein, Biotechnol. J. 7 (2012) 1242–1255.
[7] I. Demongodin, N. Sauer, L. Truffet, Performance evaluation in manufacturing systems, in: M. Diaz (Ed.), Petri Nets, Fundamental Models, Verifcatrion and Applications, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2009, pp. 527–578.
[8] A. Jungbauer, Chromatographic media for bioseparation, J. Chromatogr. A 1065 (2005) 3–12.
[9] V. Wong, R.A. Shalliker, G. Guiochon, Evaluation of the uniformity of analytical-size chromatography columns prepared by the downward packing of particulate slurries, Anal. Chem. 76 (2004) 2601–2608.
[10] M.B. Baru, A.V. Danilov, I.V. Vagenina, Size exclusion chromatography on soft and semi-rigid packing materials in the dynamic axial compression mode, J. Biochem. Biophys. Methods 57 (2003) 115–142.
[11] A.V. Danilov, L.G. Mustaeva, I.V. Vagenina, M.B. Baru, Size-exclusion chromatography on soft packing material under axial compression, J. Chromatogr. A 732 (1996) 17–25.
[12] I.V. Vagenina, E.A. Kozlovsky, L.G. Mustaeva, E.Y. Gorbunova, M.B. Baru, Size exclusion chromatography of polypeptides on Bio-Gel P2 in the dynamic axial compression mode, J. Chromatogr. A 840 (1999) 281–288.

[13] D.E. Cherrak, M. Al-Bokari, E.C. Drumm, G. Guiochon, Behavior of packing materials in axially compressed chromatographic columns, J. Chromatogr. A 943 (2002) 15–31.
[14] D.E. Cherrak, G. Guiochon, Phenomenological study of the bed-wall friction in axially compressed packed chromatographic columns, J. Chromatogr. A 911 (2001) 147–166.
[15] G. Guiochon, T. Farkas, H. Guan-Sajonz, J.H. Koh, M. Sarker, B.J. Stanley, T. Yun, Consolidation of particle beds and packing of chromatographic columns, J. Chromatogr. A 762 (1997) 83–88.
[16] J.H. Koh, B.S. Broyles, H. Guan-Sajonz, M.Z.C. Hu, G. Guiochon, Consolidation and column performance of several packing materials for liquid chromatography in a dynamic axial compression column, J. Chromatogr. A 813 (1998) 223–238.
[17] J.H. Koh, G. Guiochon, Effect of the column length on the characteristics of the packed bed and the column efficiency in a dynamic axial compression column, J. Chromatogr. A 796 (1998) 41–57.
[18] M. Kamiński, J. Klawiter, J.S. Kowalsczyk, Investigation of the relationship between packing methods and efficiency of preparative columns. II. Characteristics of the slurry method of packing chromatographic columns, J. Chromatogr. A 243 (1982) 225–244.
[19] S.C. Siu, C. Chia, Y. Mok, P. Pattnaik, Packing of large-scale chromatography columns with irregularly shaped glass based resins using a stop-flow method, Biotechnol. Progr. 30 (2014) 1319–1325.
[20] J. Klawiter, M. Kamiński, J.S. Kowalczyk, Investigation of the relationship between packing methods and efficiency of preparative columns. I. Characteristics of the tamping method for packing preparative columns, J. Chromatogr. A 243 (1982) 207–224.
[21] O. Kaltenbrunner, P. Watler, S. Yamamoto, Column qualification in process ion-exchange chromatography, Progr. Biotechnol. (2000) 201–206.
[22] A.S. Rathore, R.M. Kennedy, J.K. O'Donnell, I. Bemberis, O. Kaltenbrunner, Qualification of a chromatographic column: why and how to do it, BioPharm Int. 16 (2003) 30–40.
[23] O. Kaltenbrunner, A. Jungbauer, S. Yamamoto, Prediction of the preparative chromatography performance with a very small column, J. Chromatogr. A 760 (1997) 41–53.
[24] N. Dyson, Digital measurementof peak areas, in: R. Smith (Ed.), RSC Chromatography Monographs, The Royal Society of Chemistry, Hertfordshire, UK, 1990, pp. 140–192.
[25] N. Dyson, Peak distortion, data sampling errors and the integrator in the measurement of very narrow chromatographic peaks, J. Chromatogr. A 842 (1999) 321–340.
[26] K. Varmuza, P. Filzmoser, Introduction to Multivariate Data Analysis in Chemometrics, CRC Press, Boca Ranton, FL, 2009.
[27] J.J. Kirkland, W.W. Yau, H.J. Stoklosa, C.H. Dilks Jr., Sampling and extra-column effects in high-performance liquid chromatography; influence of peak skew on plate count calculations, J. Chromatogr. Sci. 15 (1977) 303–316.
[28] J.C. Giddings, Reduced plate height equation: a common link between chromatographic methods, J. Chromatogr. A 13 (1964) 301–304.
[29] J.J. Stickel, A. Fotopoulos, Pressure-flow relationships for packed beds of compressible chromatography media at laboratory and production scale, Biotechnol. Progr. 17 (2001) 744–751.
[30] J.T. McCue, D. Cecchini, K. Hawkins, E. Dolinski, Use of an alternative scale-down approach to predict and extend hydroxyapatite column lifetimes, J. Chromatogr. A 1165 (2007) 78–85.
[31] M.R. Schure, R.S. Maier, How does column packing microstructure affect column efficiency in liquid chromatography? J. Chromatogr. A 1126 (2006) 58–69.
[32] B.J. Stanley, C.R. Foster, G. Guiochon, On the reproducibility of column performance in liquid chromatography and the role of the packing density, J. Chromatogr. A 761 (1997) 41–51.